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14. ABSTRACT

The use of lasers by both the military and civilian community is rapidly expanding. Thus, the potential for laser eye injury and retinal damage is increasing. Sensitive and accurate methods to evaluate and follow laser retinal damage are needed. The multifocal electroretinogram (mfERG) has the potential to meet these criteria. In this study, the mfERG was used to evaluate changes to retinal function following laser exposure. Landolt C contrast acuity was also measured in the six behaviorally trained Rhesus monkeys. The monkeys then received Nd:YAG laser lesions (1064 nm, 9 ns pulse width) in each eye. One eye received a single foveal lesion of approximately 0.13 mJ total intraocular exposure (TIE) and the other received six parafoveal lesions which varied in TIE from 0.13 to 4 mJ. mfERGs and behavioral data were collected both pre- and post-exposure. mfERGs were recorded using stimuli that contained 103, 241, and 509 hexagons. Landolt C contrast acuity was measured with five sizes of Landolt C (0.16 to 5.60 cycles/degree) of varying contrast presented on a monitor at 80 cm. mfERG response densities were sensitive to the functional retinal changes caused by the laser insult. In general, larger lesions showed greater mfERG abnormality than smaller. Deficits in contrast acuity were found to be more severe in the eyes with foveal injuries. Although the mfERG and contrast acuity access different levels of the visual system, both are sensitive to laser-induced retinal damage and may compliment tests for laser eye injury triage.

15. SUBJECT TERMS
laser, lesion, retina, spatial frequency, contrast sensitivity, multifocal electroretinogram, non-human primate

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INTRODUCTION

Concurrent with the technology advances of the past decades is and increase in the manufacture and use of lasers. While lasers serve as important tools, they have the potential to cause human injuries, especially to the eyes[1-4]. Unfortunately, laser eye injuries involving the retina can be difficult to treat medically and can have devastating effects on vision. Unfortunately, in many cases it is difficult to diagnose laser retinal injuries unless central vision is affected.

The multifocal electroretinogram (mfERG) is a relatively new technique[5] in ocular electrophysiology that may provide a sensitive method to evaluate laser retinal injuries and treatment of these injuries. The mfERG provides hundreds of focal measurements from the central retina. Importantly, the measurements are objective and provide functional data in response to light. Specific response parameters can be interpreted with respect to the layer of the retina that is affected by disease or injury[6]. Numerous authors have reported changes in mfERG responses in non-human primates (NHPs) following laser retinal injury[7-10]. The mfERG has also been shown to be sensitive to many human diseases that affect the retina including diabetes[11, 12], glaucoma[13, 14], retinitis pigmentosa[15, 16], and age-related macular degeneration[16, 17].

The gold standards for diagnosing retinal changes from disease and injury are ophthalmoscopy and subjective tests of visual function (e.g., visual acuity and contrast sensitivity). Some studies have shown contrast sensitivity to be more indicative of visual disability than visual acuity in human eye diseases[18, 19]. This is because most visual acuity tests contain high contrast letters and the visual disability becomes more evident with lower contrast. Another test, contrast acuity, combines the contrast sensitivity and visual acuity tests. In contrast acuity, letters are presented on a chart and the contrast of the letters is systematically reduced from the top to bottom of the chart. A variation of the contrast acuity uses only the letter "C". Both the contrast and gap size of the "C" is varied to determine threshold contrast acuities. Importantly, NHPs can be trained to effectively accomplish subjective tests of visual acuity, contrast sensitivity, and contrast acuity.[20]

This report summarizes the efforts accomplished in the final year of the protocol. Six NHPs served as subjects for mfERG recordings before and after the instillation of small laser lesions in the retina. The same NHPs were also behaviorally trained to accomplish contrast acuity testing and, as above, testing was accomplished before and after the instillation of retinal lesions with a laser.

BODY

Statement of Work Experiment 1

Determine the sensitivity of the mfERG to detect laser lesions in the foveal and parafoveal regions of the retina.

Research Accomplishments Experiment 1

Prior experiments from this laboratory have used mfERG stimuli of 103 and 509 hexagons for pre- and post-laser lesion recordings[8, 21]. For this year's Experiment 1, 103, 241, and 509 element stimuli were used for mfERG recordings. Pre-exposure mfERGs were recorded on six NHPs. The subjects then had laser lesions applied to the retina of each eye. One small foveal lesion was applied to the retina of the non-dominant eye and six parafoveal lesions were applied to the retina of the dominant eye. The dominant eye was determined by behavior testing with the eye with the best vision judged to be the dominant eye (see Experiment 2). Laser lesions were applied with an infrared, 1064 nm laser because of the common use of this wavelength in military operations. Post-lesion mfERGs were recorded one hour, five days, three weeks, and seven weeks after laser exposure. The results of Experiment 1[22] were presented at the 2006 SPIE – The International Society for Optical Engineering conference in San Jose, California (see Appendix 1). The mfERG was found to be sensitive to the foveal lesions in five of the six exposed eyes. However, it was not as sensitive to the parafoveal lesions with only two of six eyes showing reduced responses. Advanced statistical analyses of the mfERG data are currently underway.

Statement of Work Experiment 2

Determine the sensitivity of behavior metrics (contrast acuity) to laser retinal damage.

Research Accomplishments Experiment 2

NHPs can be trained to perform tasks of visual performance such as visual acuity and contrast sensitivity[20, 23]. In the current experiment, six Rhesus monkeys were trained to perform a contrast acuity task using a modified Landolt "C" test. Monocular testing was accomplished by placing a soft, neoprene mask over one eye. Five gap sizes of the "C" (0.33, 0.98, 3.98, 6.31, and 11.15 cycles/degree) were presented at various contrast levels. The percent correct data were fit with a Probit function[24] and the 50% correct point was used as the threshold contrast for each "C" gap size. The NHPs were trained for approximately three months prior to pre-exposure testing. Pre-exposure testing was conducted until at least ten sessions were completed. Post-exposure testing was conducted for ten weeks. Figure 6 in Appendix 1 shows typical pre- and post-exposure contrast acuity results. Overall, the contrast acuity results were not sensitive to the retinal laser injuries with only two of six with foveal lesions and two of six with parafoveal lesions showing reduced post-exposure contrast acuities. The reason for the insensitivity of these measurements is unclear and the data are being reexamined.

KEY RESEARCH ACCOMPLISHMENTS

- Six NHPs behaviorally trained to complete a contrast acuity test of visual function and pre- and post-exposure testing completed.
- Pre- and post-exposure mfERGs recorded on six NHPs further validating this technique as a valuable research tool.

- Foveal and parafoveal laser lesions applied to the retinas of six NHPs. The lesions were small and did not affect the subject's ability to function. All NHPs were returned to the colony after completion of testing.
- Video and still retinal photographs were obtained on each subject during application of the laser lesions and during each mfERG recording session.

REPORTABLE OUTCOMES

Publications

C. D. DiCarlo, G. L. Martinsen, T. Garza, A. Grado, J. Morin, A. Brown, D. Stolarski, and C. Cain, "Functional and behavioral metrics for evaluating laser retinal damage," presented at Photonic Therapeutics and Diagnostics II, 2006.

Presentations

C. D. DiCarlo, G. L. Martinsen, T. Garza, A. Grado, J. Morin, A. Brown, D. Stolarski, and C. Cain, "Functional and behavioral metrics for evaluating laser retinal damage," presented at Photonic Therapeutics and Diagnostics II, 2006.

G. L. Martinsen, C. D. DiCarlo, T. Garza, A. Grado, J. Morin, A. Brown, D. Stolarski, and C. Cain, "Focal Electroretinogram Changes Following Laser Retinal Damage" 26th Annual Lasers on the Modern Battlefield Conference, Brooks City-Base, TX, 01 March 2006.

CONCLUSIONS

The use of lasers on the modern battlefield is expanding. Accompanying this expansion is the increased risk of retinal eye injuries from lasers. Thus, new ways of diagnosing laser eye injuries and evaluating laser eye injuries are needed. Research under this protocol from this year and prior years has evaluated the utility of using the mfERG to diagnose and follow treatments of laser eye injuries[8, 9, 21, 22, 25]. Unfortunately, this year's testing of NHPs contrast acuity did not find it to be a reliable diagnostic tool for laser eye injuries.

REFERENCES

1.   A. B. Thach, "Laser injuries of the eye," *Int Ophthalmol Clin*, vol. 39, pp. 13-27, 1999.
2.   J. K. Luttrull and J. Hallisey, "Laser pointer-induced macular injury.," *American Journal of Ophthalmology*, vol. 127, pp. 95-96, 1999.
3.   Y. Barkana and M. Belkin, "Laser eye injuries," *Survey of Ophthalmology*, vol. 44, pp. 459-478, 2000.
4.   D. J. Lund, P. R. Edsall, D. R. Fuller, and S. W. Hoxie, "Ocular Hazards of Tunable Continuous-Wave Near-Infrared Laser Sources," presented at Progress in Biomedical Optics (BiOS), San Jose, CA, 1996.
5.   E. E. Sutter and D. Tran, "The field topography of ERG components in man - I. The photopic luminance response," *Vision Research*, vol. 32, pp. 433-446, 1992.
6.   D. C. Hood, L. J. Frishman, S. Saszik, and S. Viswanathan, "Retinal origins of the primate multifocal ERG: implications for the human response," *Invest Ophthalmol Vis Sci*, vol. 43, pp. 1673-85, 2002.
7.   H. Zwick, B. E. Stuck, A. Akers, P. Edsall, C. D. DiCarlo, and D. J. Lund, "Multifocal electroretinogram (MFERG) evaluation of laser-induced secondary damage in the non-human primate (NHP)," presented at Ophthalmic Technologies XV, 2005.
8.   C. D. DiCarlo, J. Brown, A. Grado, J. Sankovich, H. Zwick, D. J. Lund, and B. E. Stuck, "Use of the multifocal electroretinogram (mfERG) for assessing the response of 670 nm light emitting diodes (LED) photoillumination in an animal model with laser retinal injuries," presented at Lasers in Surgery: Advanced Characterization, Therapeutics, and Systems XIV, 2004.
9.   C. D. DiCarlo, H. D. Hacker, A. Brown, R. Cheramie, G. L. Martinsen, B. Rockwell, and B. E. Stuck, "Retinal, functional, and morphological comparisons of two different macaque species, Macaca mulatta and Macaca fasicularis, for models of laser eye injury," presented at Photonic Therapeutics and Diagnostics, 2005.
10.   D. Hood, L. Frishman, S. Saszik, S. Viswanathan, and J. Robson, "The variations in the waveform of the primate cone ERG with retinal eccentricity depend upon inner and outer retinal contributions [ARVO Abstract]," *Investigative Ophthalmology and Visual Science*, vol. 41, pp. S496. Abstract nr 2639, 2000.
11.   B. Fortune, M. E. Schneck, and A. J. Adams, "Multifocal electroretinogram delays reveal local retinal dysfunction in early diabetic retinopathy," *Investigative Ophthalmology and Visual Science*, vol. 40, pp. 2638-51, 1999.
12.   A. M. Palmowski, E. E. Sutter, J. Marcus A Bearse, and W. Fung, "Mapping of retinal function in diabetic retinopathy using the multifocal electroretinogram," *Investigative Ophthalmology and Visual Science*, vol. 38, pp. 2586-2596, 1997.
13.   M. A. Bearse and E. E. Sutter, "Detection of functional abnormalities in glaucoma using a new multifocal ERG paradigm [ARVO Abstract]," *Investigative Ophthalmology and Visual Science*, vol. 41, pp. S103. Abstract nr 536, 2000.
14.   D. C. Hood, X. Zhang, V. C. Greenstein, S. Kangovi, J. G. Odel, J. M. Liebmann, and R. Ritch, "An interocular comparison of the multifocal VEP: a possible

technique for detecting local damage to the optic nerve," *Invest Ophthalmol Vis Sci*, vol. 41, pp. 1580-7, 2000.

15. D. C. Hood, K. Holopigean, V. Greenstein, W. Seiple, J. Li, E. E. Sutter, and R. E. Carr, "Assessment of local retinal function in patients with retinitis pigmentosa using the multi-focal ERG technique.," *Vision Research*, vol. 38, pp. 163-179, 1997.

16. M. A. Bearse and E. E. Sutter, "Imaging localized retinal dysfunction with the multifocal electroretinogram," *Journal of the Optical Society of America A*, vol. 13, pp. 634-642, 1996.

17. J. Li, M. O. Tso, and T. T. Lam, "Reduced amplitude and delayed latency in foveal response of multifocal electroretinogram in early age related macular degeneration," *Br J Ophthalmol*, vol. 85, pp. 287-90., 2001.

18. D. B. Elliott, M. A. Hurst, and J. Weatherill, "Comparing clinical tests of visual function in cataract with the patient's perceived visual disability," *Eye*, vol. 4, pp. 712-7, 1990.

19. G. Lennerstrand and C. O. Ahlstrom, "Contrast sensitivity in macular degeneration and the relation to subjective visual impairment," *Acta Ophthalmologica*, vol. 67, pp. 225-33, 1989.

20. J. Rhodes and P. Garcia, "Grating visual acuity following hemorrhagic foveal lesions," *Lasers and Light*, vol. 7, pp. 153-165, 1996.

21. C. D. DiCarlo, H. Zwick, H. Rentmeister-Bryant, J. M. Sankovich, A. Brown, A. Grado, R. J. Dicks, A. Akers, and B. E. Stuck, "Multifocal electroretinography: a functional laser injury metric," presented at Laser and Noncoherent Light Ocular Effects: Epidemiology, Prevention, and Treatment III, 2003.

22. C. D. DiCarlo, G. L. Martinsen, T. Garza, A. Grado, J. Morin, A. Brown, D. Stolarski, and C. Cain, "Functional and behavioral metrics for evaluating laser retinal damage," presented at Photonic Therapeutics and Diagnostics II, 2006.

23. S. J. Fairhall, C. A. Dickson, L. Scott, and P. C. Pearce, "A non-invasive method for studying an index of pupil diameter and visual performance in the rhesus monkey," *J Med Primatol*, vol. 35, pp. 67-77, 2006.

24. D. J. Finney, *Probit analysis*, 3rd ed. Cambridge: Cambridge University Press, 1971.

25. C. D. DiCarlo, J. Brown, H. D. Hacker, R. Cheramie, S. Schuschereba, L. Valo, D. R. Clarkson, J. Sankovich, H. Zwick, D. J. Lund, and B. E. Stuck, "Effect of light emitting diode (LED) therapy on the survival of photoreceptors following argon laser injury," presented at Ophthalmic Technologies XV, 2005.

Functional and behavioral metrics for evaluating laser retinal damage

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ABSTRACT

The use of lasers by both the military and civilian community is rapidly expanding. Thus, the potential for and severity of laser eye injury and retinal damage is increasing [1]. Sensitive and accurate methods to evaluate and follow laser retinal damage are needed. The multifocal electroretinogram (mfERG) has the potential to meet these criteria. In this study, the mfERG was used to evaluate changes to retinal function following laser exposure. Landolt C contrast acuity was also measured in the six behaviorally trained Rhesus monkeys. The monkeys then received Nd:YAG laser lesions (1064 nm, 9 ns pulse width) in each eye. One eye received a single foveal lesion of approximately 0.13 mJ total intraocular exposure (TIE) and the other received six parafoveal lesions which varied in TIE from 0.13 to 4 mJ. mfERGs and behavioral data were collected both pre- and post-exposure. mfERGs were recorded using stimuli that contained 103, 241, and 509 hexagons. Landolt C contrast acuity was measured with five sizes of Landolt C (0.33 to 11.15 cycles/degree) of varying contrast. mfERG response densities were sensitive to the functional retinal changes caused by the laser insult. In general, larger lesions showed greater mfERG abnormalities than smaller laser lesions. Deficits in contrast acuity were found to be more severe in the eyes with foveal injuries. Although the mfERG and contrast acuity assess different areas of the visual system, both are sensitive to laser-induced retinal damage and may be complementary tests for laser eye injury triage.

Keywords: multifocal electroretinogram, mfERG, laser retinal injury, nonhuman primate, contrast sensitivity, visual acuity

1. INTRODUCTION

The use of lasers on the modern battlefield is expanding. Military uses of lasers include target range determination, target designation, illumination and high-energy weapons among others. Most of these lasers are of infrared (IR) or visible wavelengths. In addition, laser illumination of commercial airline pilots has been reported in recent years[2-4]. Thus far, all reports have indicated that commercial airline illuminations have been with visible lasers. Whether from military or civilian sources, lasers of these wavelengths, IR and visible, can cause serious injury to the eye, specifically the retina. The severity of a retinal injury depends on a number of factors affiliated with the power of the laser and the distance from the laser when illuminated, such as the area of the retina illuminated, the energy absorbed by the retina, and the wavelength and pulse duration of the laser. A small injury to the peripheral retina might go unnoticed to the exposed person and would not likely have any serious complications that would compromise vision in the future. Conversely, a large lesion to the fovea can have immediate and dire consequences for vision[5-7].

There are two primary ways to determine if a person has had a retinal injury after exposure to a laser. The first is based on the patient's symptoms that might include seeing floaters, reduced acuity, and Amsler grid distortion. The second way to diagnose a laser-caused retinal injury is via ophthalmoscopy. Examination of an injured retina with an

ophthalmoscope might reveal retinal lesions, retinal scars, retinal hemorrhage, and possibly vitreous hemorrhage. The multifocal electroretinogram (mfERG) is a relatively new technique that objectively tests focal function of the central retina. Using a pseudorandom flickering on a monitor, hundreds of responses from the central retina can be recorded[8, 9] .The mfERG has been shown to be sensitive to retinal changes that occur in many diseases including age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, and retinal detachment[10]. mfERG response characteristics have been shown to vary depending on the part of the retina that is affected by a disease or injury [11] . The mfERG therefore has the potential to be a sensitive and objective method to assess patients who have sustained laser eye injury.

In the current study, we tested contrast acuity and recorded mfERGs in nonhuman primates before and after exposing each eye with an Nd:YAG (1064 nm) laser. Results are presented and the potential for using the mfERG to evaluate laser retinal injuries is discussed.

2. METHODS

2.1 Animal Preparation: Six Rhesus monkeys (8.0 – 11.5 kg) housed under standard laboratory conditions (12 hours light/12 hours dark) were used in this study. All animals involved in this study were procured, maintained, and used in accordance with Army Regulation 40-33, the Federal Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the institute of Laboratory Animal Resources, National Research Council. The Air Force Research Laboratory (AFRL) at Brooks City-Base, Texas, has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, (AAALAC) since 1967. Using a standard protocol reported elsewhere [12, 13], all animals received local (bupivacaine/lidocaine peribulbar injections) and general anesthesia (propofol via syringe pump) to stabilize eye movements for the laser exposures and for mfERG recordings.

2.2 Laser Exposures: All animals received one laser exposure of approximately 0.11 to 0.32 mJ total intraocular exposure (TIE) to the fovea of the non-dominant eye (as determined by prior contrast acuity testing). The other eye received six parafoveal lesions which varied in TIE from 0.13 to 4 mJ depending on location. (see Table 1). Exposure numbers (Exp #) 1 and 2 were applied inferior to the fovea, 3 and 4 temporal to the fovea, and 5 and 6 superior to the fovea. Exp # 7 was applied to the fovea of the other eye. One animal (D26Z) received an additional four test lesions inferior to the fovea prior to applying the six parafoveal lesions. A vitreous hemorrhage was caused by one of the test lesions but the hemorrhage remained in the inferior vitreous during the follow-up (Day 0) mfERG testing. In addition, a vitreous hemorrhage was caused in one animal (D10Z) by the application of the foveal lesion.

Table 1.

Exp #	Average TIE (mJ)	Standard Deviation (mJ)	Retinal Location
1	3.58	0.53	Parafovea (inf)
2	3.79	0.38	Parafovea (inf)
3	0.34	0.03	Parafovea (temp)
4	0.33	0.03	Parafovea (temp)
5	0.19	0.06	Parafovea (sup)
6	0.19	0.06	Parafovea (sup)
7	0.21	0.10	Fovea

2.3 Multifocal Electroretinogram Recordings: Baseline and post-laser exposure mfERGs were recorded on the animals on Day 0 (about one hour after laser exposure), Day 5, Week 3, and Week 7. Stimulus arrays of 103, 241, and 509 unscaled hexagons were used for recordings at each session. Only the 241 hexagon results are presented here (Fig. 1). Custom manufactured Burian-Allen electrodes (Hansen Ophthalmic Development Lab, Coralville, IA) were placed on the anesthetized (proparacaine drops) corneas of the anesthetized animal subjects for the recording process. mfERGs were collected from the subjects using the Visual-Evoked Response Imaging System (VERIS™, Electro-Diagnostic Imaging, Redwood City, CA). An infrared camera allowed for continuous visualization of the posterior segment of the eye during mfERG recordings. The VERIS system stimulus pattern was projected onto the fundus and the optics were

located approximately 4 cm from the cornea. Using unscaled stimuli, 103, 241, and 509 focal ERG response recordings were recorded from each eye during each session. The luminance of a hexagon when white was approximately 300 cd/m^2 and when black was as dark as possible yielding a contrast of nearly 100%. Using the VERIS system software for artifact removal, filtering, and the combining of left and right eyes, normal files were created for each animal using that animal's baseline 241 hexagon recordings. With these normal files, mfERG response density plots were evaluated both pre- and post-laser exposure and any signs of deviation from normal noted.

2.3 Behavioral Testing: Numerous pre- and post-laser exposure contrast acuity measurements were collected on each animal. After initial acclimation to the test environment, the animals were trained on a series of tasks that were of increasing complexity over a series of several months. The animal was elevated to the next most difficult task once the animal had achieved an accuracy of approximately 90% for the less difficult task. The final pre-exposure acuity/contrast sensitivity measurements were recorded after approximately two months of training on this most difficult task level. For each acuity measurement session, an animal was placed in a chair 80 centimeters (cm) from a display monitor. A soft, neoprene mask was placed over the animal's eyes and used to occlude the opposite eye for monocular recordings. Five gap sizes of a Landolt "C" (0.33, 0.98, 3.98, 6.31, and 11.15 cycles/degree) were tested and a psychophysical staircase (gradual lightening of the "C" with a reduction in size) was used to control the contrast. Animals held down a lever until they saw the "C" at which point they released the lever. Correct responses received within 5 seconds were rewarded. A Probit [14] function (frequency of seeing curve or "S-shaped" curve) was fit to the percent correct data for each "C" size, for each animal, and for each session. The 50% correct point on the Probit function was used as the threshold contrast for each "C" size.

3. RESULTS

Figure 2 shows the TIE applied to animal D26Z's right (foveal) and left (6 parafoveal and 4 test lesions) retinas. The drawings illustrate the approximate location of the lesions and the photographs show the post-exposure appearance of the lesions. The right fovea received a single laser exposure to the fovea of very low energy and the lesion is barely visible on the retinal photograph. One of the test lesions applied to the inferior retina of the left eye (OS1) caused an immediate vitreous hemorrhage. The parafoveal lesions in the left eye can be seen in the second OS photograph (OS2).

The baseline mfERG 3D response density plot from D26Z's left eye is shown in Figure 3. Response density is essentially response amplitude. Large changes in waveform timing can affect response density. The top, lower left and lower right plots are referred to as the "Difference," the "Subject," and the "Reference" plots, respectively. The view is a "Field" view which has the superior aspect of the retina on the bottom of the image and the inferior aspect of the retina on the top half of the image. The subject plot (Fig. 3 lower left) shows the animal's baseline response. An area with reduced responses showing the optic disc (blind spot) is visible in the left, slightly inferior portion of the subject plot. The reference plot (bottom right) is composed of baseline recordings from both eyes. The reference plot is somewhat bumpy reflecting the fact that the subject's baseline recording from the right eye was somewhat noisy. This is also reflected in the difference plot (upper) that appears to have some areas of depression, especially in the fovea. This is considered the normal condition for these baseline and reference recordings.

Figure 4 shows D26Z's five day post-exposure mfERG recording. The subject plot shows greater central depression compared with the baseline and the small foveal peak seen in the baseline plot is gone. The area where the test lesions were administered and vitreous hemorrhage occurred (inferior retina/superior field) shows a large area with reduced responses (Fig. 4, "Difference" plot).

Figure 5 shows the baseline and follow-up mfERG recording "Difference" plots for D26Z. Although D26 Z received only a small, barely visible lesion to the right fovea, all follow-up plots show reduced response densities centrally compared with the combined normals files. The left eye responses are reduced in the inferior aspect of the retina (superior aspect of plot on a field view) corresponding to the area where the test lesions were applied, although there appears to be recovery in the later recordings (D. and E.).
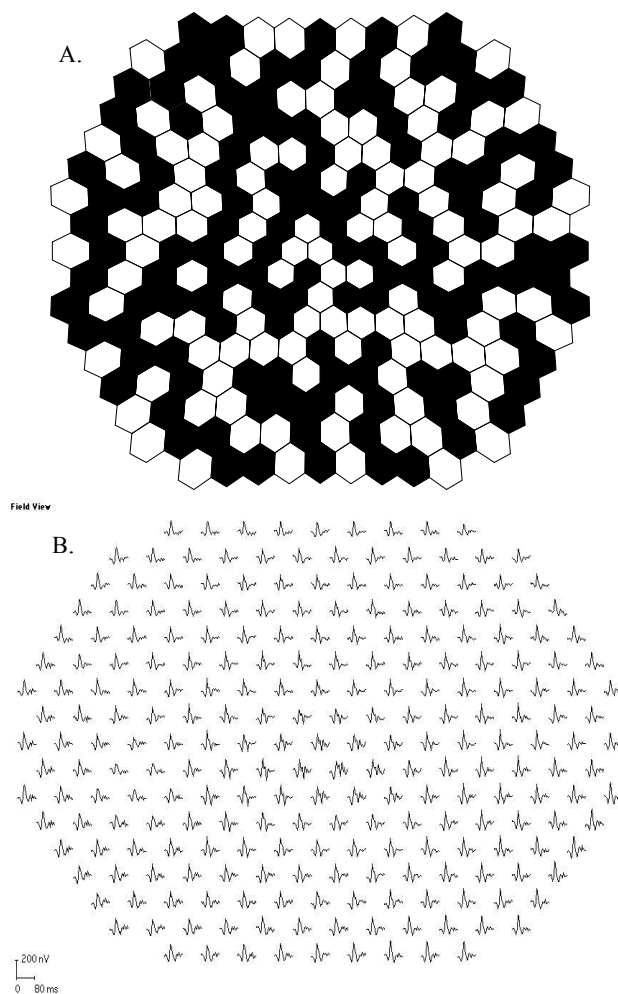
A.

Field View

B.

Figure 1. (A) mfERG stimulus of 241 hexagons. The presentation of each hexagon was controlled by a pseudorandom sequence and each had a 50% probability of being light and dark. (B) Representative mfERG waveforms from an uninjured Rhesus monkey retina stimulated with a 241 element stimulus.

Treatment date: 5/5/2005; D26Z;

1064 nm laser, 8 - 9 ns pulse width

Exp #	Power (calc)		
1, 2	3.78, 3.9	mJ	
3, 4	0.32, 0.32	mJ	
5, 6	0.16, 0.16	mJ	
7	0.11	mJ	
Test Shots	Power		
T1	3.41	mJ	no immed lesion
T2	4.27	mJ	no immed lesion
T3	6.28	mJ	Hemorrhage
T4	0.32	mJ	

OD   OS

OD   OS1   OS2
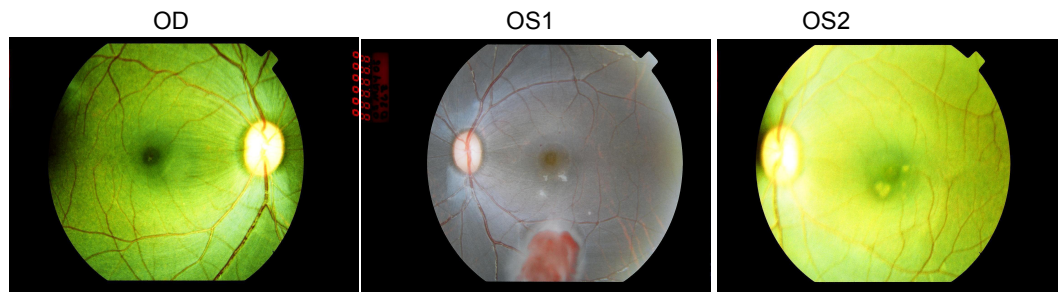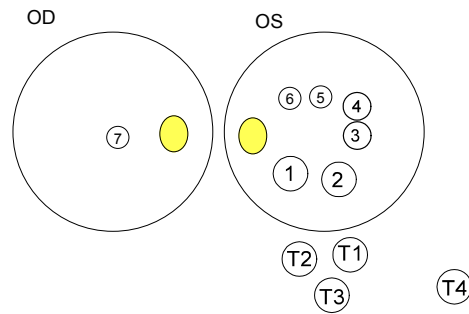
Figure 2. Laser TIEs to the right eye (#7) and left eye (#1 - #6). Four test lesions were applied to the inferior left retina. The drawings show the location of the lesions and the photographs show post-exposure lesions.

Field View   Difference

Field View   Subject

Field View   Reference
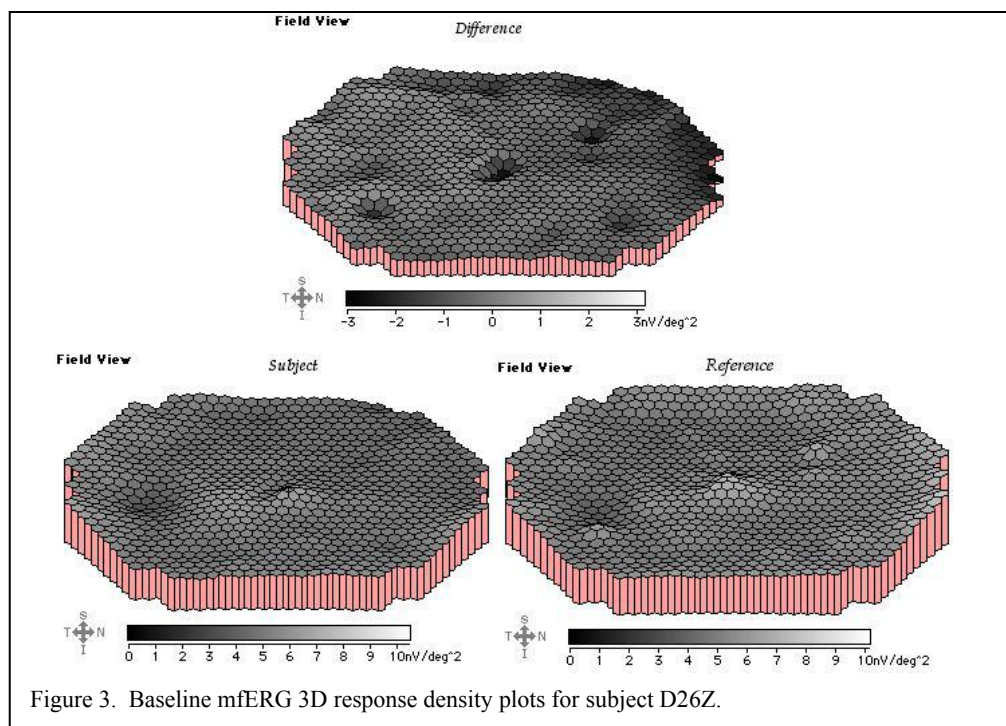
Figure 3. Baseline mfERG 3D response density plots for subject D26Z.
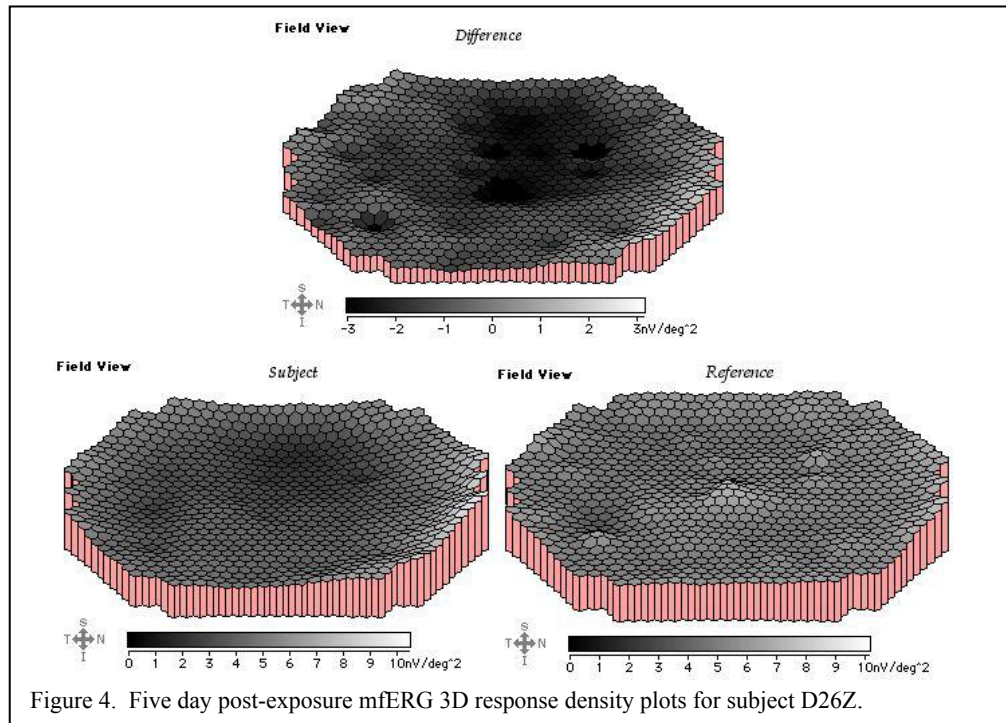
Figure 4. Five day post-exposure mfERG 3D response density plots for subject D26Z.

The average behavioral results for D26Z are shown in Figure 6. For each eye, the post-exposure results are reduced compared with the pre-exposure results (Paired T-test; $p < 0.05$). Generally, post-exposure responses are reduced more at low- and mid-spatial frequencies and are relatively normal at the highest spatial frequency. Although on average D26Z's post-exposure responses were reduced, individual responses were not always reduced. Overall for the right eye, 10 of 13 individual recordings were reduced at all spatial frequencies. For the left eye, 10 of 12 post-exposure measurements were reduced at all spatial frequencies. Thus, on an individual basis, some of D26Z's behavioral results do not show a deficit at some or all spatial frequencies.

Figure 7 shows the five day post-exposure laser lesions from D10Z. A large vitreous hemorrhage was caused by the foveal laser exposure to the left eye and the lesion can be clearly seen in the right eye. The 6 parafoveal lesions in the left eye are clearly visible in the retina. The mfERG response density difference plots for D10Z at baseline, 5 day post-exposure, and 3 week post-exposure are shown in Figure 8. The large foveal lesion in the right eye is clearly evident in the post-exposure plot. However, the large parafoveal lesions that are evident in the photograph of the left eye are not easily seen in the mfERG responses.

Despite having a large foveal lesion and reduced mfERG responses, D10Z's contrast acuity was significantly better after the laser lesions were applied (Fig. 9). Post-exposure behavioral results were generally better than pre-exposure results.

Overall, five of six subjects that received foveal laser exposures showed reduced central responses on their mfERG results. Only two of six subjects with parafoveal lesions showed definite mfERG reductions. For the behavioral results, only two of six animals who received foveal lesions had statistically reduced average contrast acuities. For animals with parafoveal lesions, two of six also displayed reduced contrast acuities on average. Individual post-exposure contrast acuity results varied widely from better to worse than pre-exposure results.
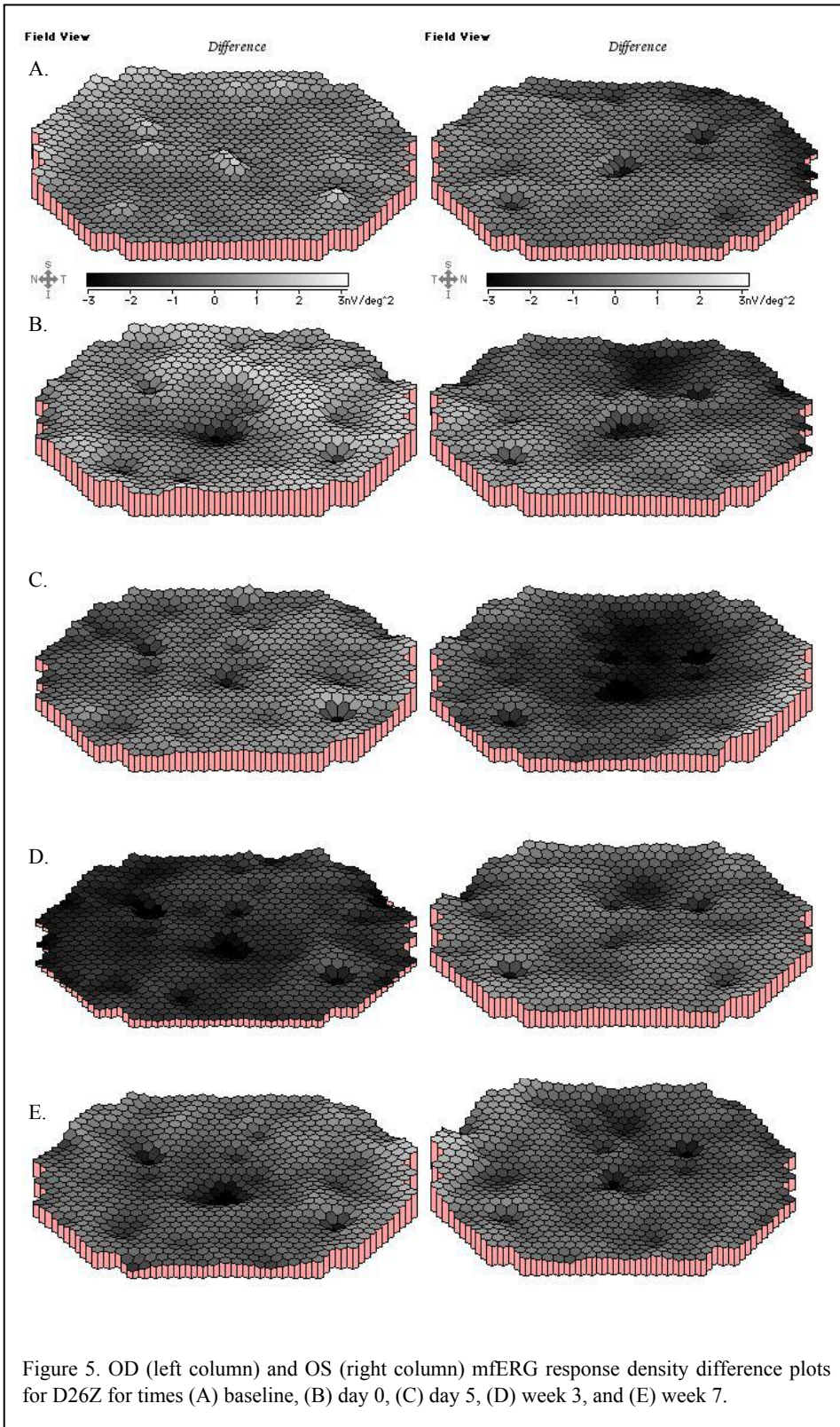
Figure 5. OD (left column) and OS (right column) mfERG response density difference plots for D26Z for times (A) baseline, (B) day 0, (C) day 5, (D) week 3, and (E) week 7.
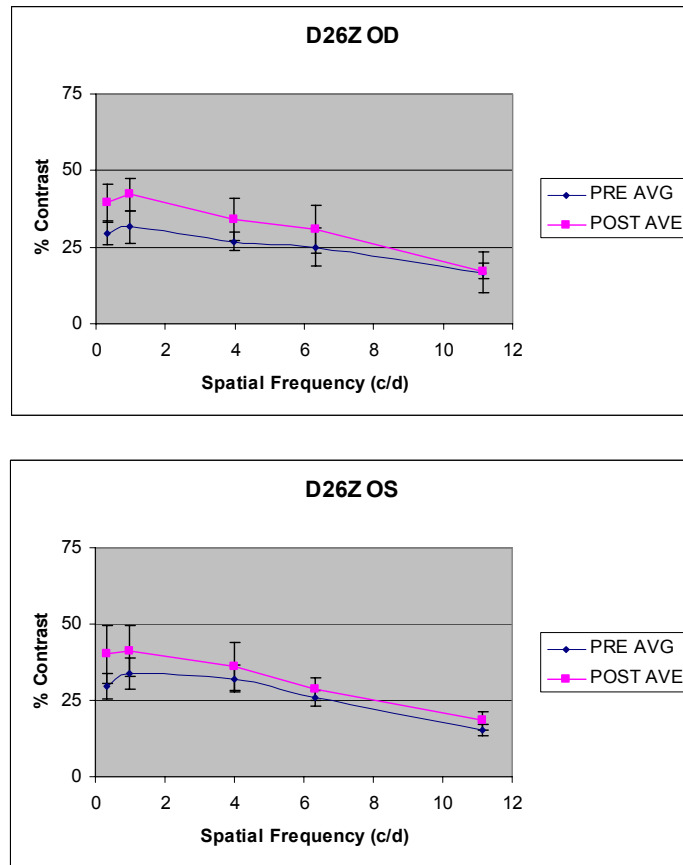
Figure 6. Average pre- and post-exposure contrast acuity results for the right eye (upper) and left eye (lower) for D26Z. Shown are results of each of the 5 "C" gap sizes. Contrast results are for the 50% correct values from the Probit functions. Error bars show +/- 1 standard deviation.
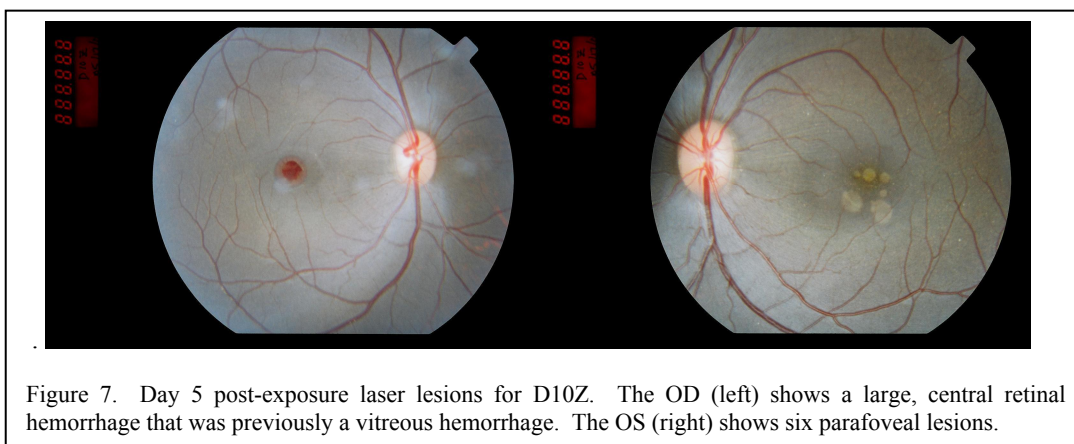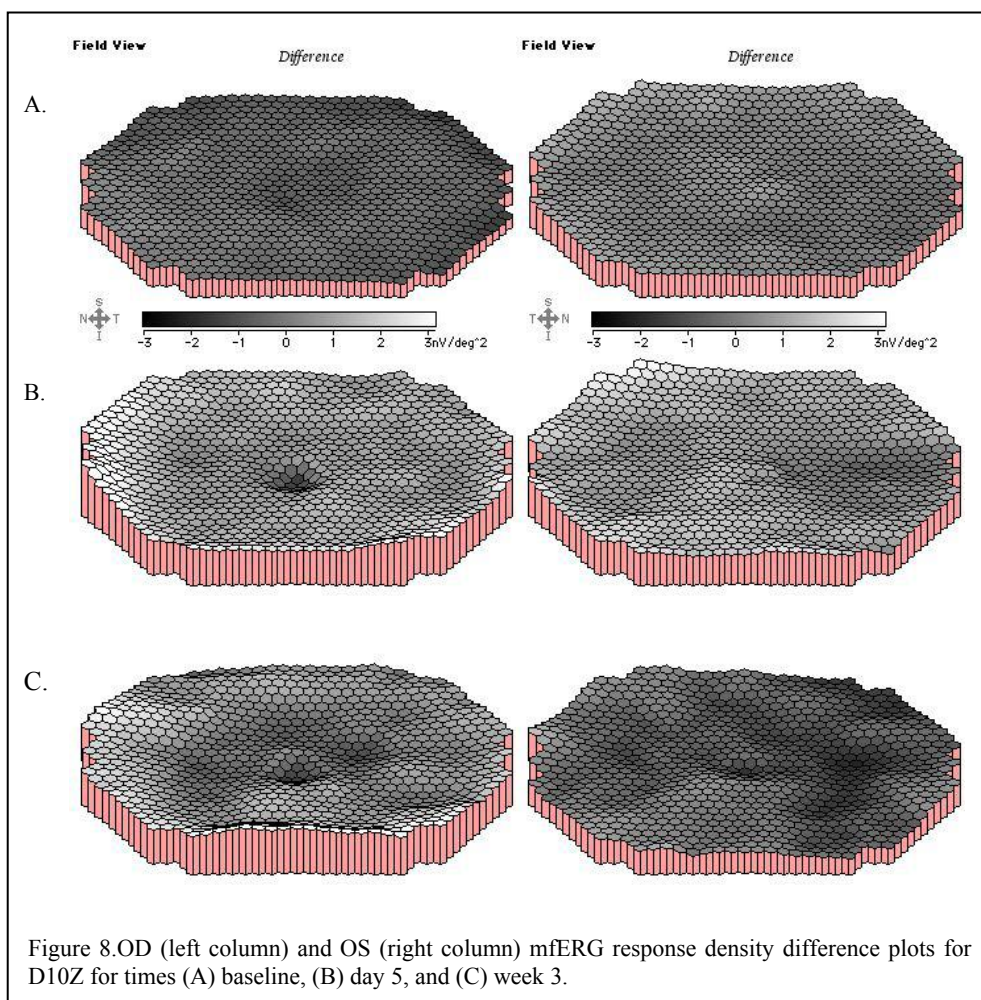

Figure 7. Day 5 post-exposure laser lesions for D10Z. The OD (left) shows a large, central retinal hemorrhage that was previously a vitreous hemorrhage. The OS (right) shows six parafoveal lesions.

Figure 8. OD (left column) and OS (right column) mfERG response density difference plots for D10Z for times (A) baseline, (B) day 5, and (C) week 3.
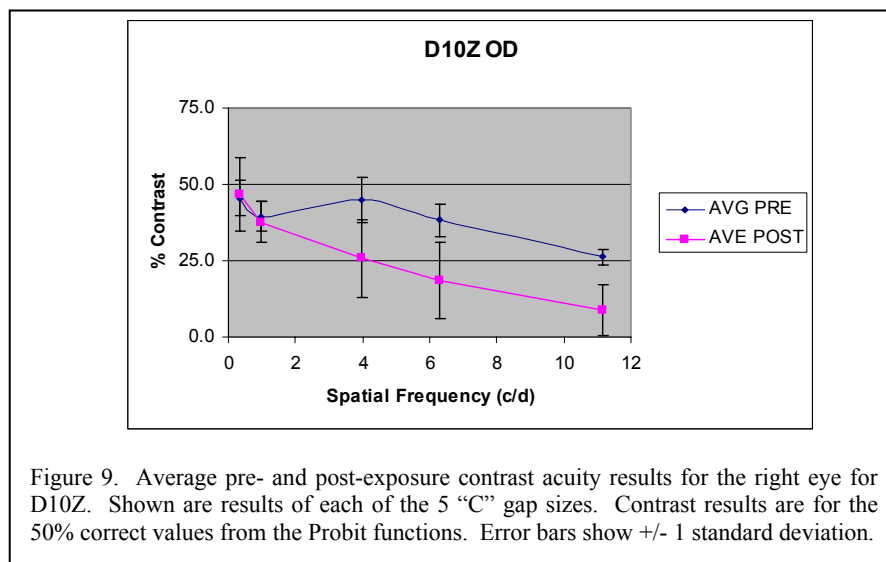

Figure 9. Average pre- and post-exposure contrast acuity results for the right eye for D10Z. Shown are results of each of the 5 "C" gap sizes. Contrast results are for the 50% correct values from the Probit functions. Error bars show +/- 1 standard deviation.

4. CONCLUSIONS

The monkey has proven to be a useful model to evaluate retinal function and changes with the mfERG[15, 16]. The mfERG may also prove valuable in detecting and following laser retinal injuries. The mfERG has been found to be sensitive to large lesions from an argon laser [17] and to secondary laser damage [7]. The current study found readily visible mfERG response reductions in four of six eyes with foveal exposures. Less definitive results were found in eyes with parafoveal lesions. Additional data exploration is required to quantify the overall sensitivity of the mfERG to detect laser retinal injuries in the nonhuman primate model.

Fewer than half of the laser treated eyes showed reduced contrast acuity after laser exposure. Individually, many post-exposure measurements produced better acuity than pre-exposure tests. The reasons for this are not clear but may include inadequacies in the testing and evaluation software, the improvement of the animal's performance to accurately complete a task over time and the ability of the visual system to fill in the retinal gaps where the damage occurred. Additionally, acuity testing in the non-human primate is a difficult task and may sometimes be too variable to produce reliable results.

5. ACKNOWLEDGEMENTS

6. REFERENCES

1.   A. B. Thach, "Laser injuries of the eye", *Int Ophthalmol Clin*, vol. 39, pp. 13-27, 1999.
2.   CNN.com, "Man quizzed about laser incidents", Atlanta, GA, 2005, pp. http://edition.cnn.com/2004/US/12/31/laser.aircraft/.
3.   LaserFocusWorld, "Are laser pointers really a hazard?" Laser Focus World, 2005, pp. http://lfw.pennnet.com/Articles/Article_Display.cfm?Section=ARTCL&SubSection=Display&PUBLICATION_ID=12&ARTICLE_ID=220602.
4.   USATODAY.com, "FBI investigates laser beam directed into airplane cockpit", Cleveland, OH: Associated Press, 2004, pp. http://www.usatoday.com/travel/news/2004-12-29-laser-beam_x.htm.
5.   H. Zwick, "Visual function changes after laser exposure", Letterman Army Institute of Research, San Francisco, technical report 84-48, June 1984.
6.   H. Zwick, K. R. Bloom, and E. S. Beatrice, "Permanent visual change associated with punctate foveal lesions", in *Colour Vision Deficiencies IX*, B. Drum and G. Verriest, Eds. Dordrecht: Kluwer Academic Publishers, 1989, pp. 251-60.
7.   H. Zwick, C. D. DiCarlo, B. E. Stuck, and D. J. Lund, "MFERG Analysis of Longterm Laser Induced Retinal Injury in the Non-Human Primate" *Invest Ophthalmol Vis Sci*, vol. 44, 2003.
8.   E. E. Sutter, "Imaging visual function with the multifocal m-sequence technique", *Vision Res*, vol. 41, pp. 1241-55, 2001.
9.   D. C. Hood, "Assessing retinal function with the multifocal technique", *Prog Retin Eye Res*, vol. 19, pp. 607-46, 2000.
10.   D. C. Hood and X. Zhang, "Multifocal ERG and VEP responses and visual fields: comparing disease-related changes", *Doc Ophthalmol*, vol. 100, pp. 115-37, 2000.
11.   D. C. Hood, V. Greenstein, L. Frishman, K. Holopigian, S. Viswanathan, W. Seiple, J. Ahmed, and J. G. Robson, "Identifying inner retinal contributions to the human multifocal ERG", *Vision Res*, vol. 39, pp. 2285-91, 1999.

12. C. D. DiCarlo, H. Zwick, H. Rentmeister-Bryant, J. M. Sankovich, A. Brown, A. Grado, R. J. Dicks, A. Akers, and B. Stuck, "Multifocal electroretinography: a functional laser injury metric", presented at SPIE/BIOS: Progress in Biomedical Optics and Imaging, San Jose, CA, 2003.

13. C. P. Cain, C. D. DiCarlo, B. A. Rockwell, P. K. Kennedy, G. D. Noojin, D. J. Stolarski, D. X. Hammer, C. A. Toth, and W. P. Roach, "Retinal damage and laser-induced breakdown produced by ultrashort-pulse lasers", *Graefes Arch Clin Exp Ophthalmol*, vol. 234, pp. S28-37, 1996.

14. D. J. Finney, *Probit Analysis*, Third Edition ed: Cambridge University Press, 1971.

15. D. C. Hood, M. A. Bearse, Jr., E. Sutter, S. Viswanathan, and L. Frishman, "The optic nerve head component of the monkey's (macaca mulatta) multifocal electroretinogram (mERG)", *Vision Res*, vol. 41, pp. 2029 - 41, 2001.

16. D. C. Hood, L. J. Frishman, S. Viswanathan, J. G. Robson, and J. Ahmed, "Evidence for a ganglion cell contribution to the primate electroretinogram (ERG): effects of TTX on the multifocal ERG in macaque", *Vis Neurosci*, vol. 16, pp. 411-6, 1999.

17. C. D. DiCarlo, J. Brown, H. D. Hacker, R. Cheramie, S. Schushereba, L. Valo, D. R. Clarkson, J. Sankovich, H. Zwick, D. J. Lund, and B. E. Stuck, "Effect of Light Emitting Diode (LED) therapy on the survival of photoreceptors following Argon laser injury", presented at Proceedings of Laser and Noncoherent Light Ocular Effects: Epidemiology, Prevention and Treatment, San Jose, CA, 2005.